The quest of anticancer vaccines: Deciphering the fine-epitope specificity of cancer-related monoclonal antibodies by combining microarray screening and STD-NMR.

Helena Coelho,[§] Takahiko Matsushita,[‡] Gerard Artigas,[‡] Hiroshi Hinou,[‡] F. Javier Cañada,[⊥] Richard Lo-Man,[†,¦] Claude Leclerc,[†,¦] Eurico J. Cabrita,[§] Jesús Jiménez-Barbero,[#] Shin-Ichiro Nishimura,[‡] Fayna Garcia-Martín,[‡,*] Filipa Marcelo[§,*]

[§]UCIBIO, REQUIMTE, Dept. de Química, Faculdade de Ciências e Tecnologia, UNL, 2829-516 Caparica, Portugal

[‡]Faculty of Advanced Life Science and Graduate School of Life Science, Hokkaido University, N21, W11, Kita-ku, Sapporo 001-0021, Japan

[⊥]Centro de Investigaciones Biológicas, CIB-CSIC, Ramiro de Maeztu 9, 28040, Madrid, Spain

[†]Unité de Régulation Immunitaire et Vaccinologie, Equipe Labellisée Ligue Contre le Cancer, Paris, Institut Pasteur, 25-28 Rue du Dr Roux, 75724 Paris Cedex 15, France

[¦]INSERM U1041, 75724 Paris Cedex 15, France.

[#]CIC bioGUNE Bizkaia Technological Park, Building 801A-1º, 48160 Derio, Spain and Ikerbasque, Basque Foundation for Science, 48005 Bilbao, Spain

Supporting Information Placeholder

ABSTRACT: The identification of MUC1 tumor-associated Tn antigen (αGalpNAc(1→*O*-Ser/Thr) has boosted the development of anticancer vaccines. Combining microarrays (MA) and saturation transfer difference NMR (STD-NMR), we have characterized the fine-epitope mapping of a MUC1 chemical library (naked and Tn-glycosylated) towards two families of cancer-related monoclonal antibodies (anti-MUC1 and anti-Tn mAbs). Anti-MUC1 mAbs clone VU-3C6 and VU-11E2 recognize naked MUC1-derived peptides and bind GalNAc in a peptide-sequence-dependent manner. In contrast, anti-Tn mAbs clone 8D4 and 14D6 mostly recognize the GalNAc and do not bind naked MUC1-derived peptides. These anti-Tn mAbs showed a clear preference for glycopeptides containing the Tn-Ser antigen rather than the Tn-Thr analogue, stressing out the role of the underlying amino acid (Ser or Thr) in the binding process. The strategy reported can be employed, in general, to unveil the key minimal structural features that modulate antigen-antibody recognition with particular relevance for the development of MUC1 anticancer vaccines.

MUC1 is a glycoprotein that shows a tandem repeating domain, with five possible *O*-glycosylation sites, of conserved 20 amino acids (HGVT*S*APDT*RPAPGS*T*APPA, asterisk shows a potential *O*-glycosylation site).[1] In normal tissues, the protein backbone carries complex oligosaccharides, with a α-*O*-GalNAc-unit directly linked to the hydroxyl group of serine (Ser) or threonine (Thr). In tumor cells, the expression of MUC1 is usually increased with aberrant glycosylation as the carbohydrate side chains are incomplete.[2] As a result, different epitopes, as the Tn antigen (α-GalNAc(1→*O*-Ser/Thr), are now exposed to the immune system and can be used to the design of synthetic MUC1-based antitumor vaccines.[3] Efforts to overcome the α-Tn low immunogenicity have been addressed based on clustered Tn-antigen mimetics.[4] As well, a therapeutic vaccine that encompasses Tn-antigen clusters and peptidic CD4+ T-cell epitopes (MAG-Tn3) recently entered in clinical trial.[5] On this basis, the elucidation of the key MUC1 antigenic elements is a matter of high interest.[6] On the one hand, the specificity of anti-MUC1 mAbs has been assigned to the chemical nature of the glycans attached to their peptide epitopes.[7] On the other hand, the density of Tn motif and the involvement of additional amino acids in the antigenic determinant namely the aglyconic part of the Tn structure (Ser *vs* Thr) are critical for anti-Tn mAbs specificity.[8,9] Despite these advances, the precise chemical epitopes of most anticancer mAbs remain uncertain. Microarray technique (MA) has arisen in recent years as a versatile platform for accomplishing massive parallel screening and processing an onset of ligand-protein comparative profile in a compact format.[10] MA are commonly used for the epitope mapping analysis of potentially therapeutic antibodies,[11,12] and its limitation to provide a deep knowledge of the binding mode makes necessary its cooperation with other experimental approaches. Furthermore, the design of accurate anticancer vaccines require the full understanding of the interactions, at the atomic level, between tumor-associated motifs and their specific antibodies. X-ray crystallography and NMR spectroscopy have definitely become the main sources of achieving structural information on ligand-receptor complexes.[13] However, the intrinsic flexibility of carbohydrate antigens may hamper a detailed X-ray analysis[14] and thus NMR methods assisted by computational calculations may permit the access to key insights into the structure and dynamic features of

Table 1. Synthetic (Glyco)peptides Used for Antibody Mapping by STD-NMR Analysis and MA Screening.

entry	(glyco)peptide[a]	entry	(glyco)peptide[b]
1	APDTRPAPGS		
2	HGVTSAPDTRPAPGSTAPPA	**2'**	GVTSAPDTRPAPGSTAPPAHGVT
3	PPAHGV SAPDTR	**3'**	GV SAPDTRPAPGSTAPPAHGVT
4	PAHGVT APDTRP	**4'**	GVT APDTRPAPGSTAPPAHGVT
5	VTSAPD RPAPGS	**5'**	GVTSAPD RPAPGSTAPPAHGVT
6	TRPAPG TAPPAH	**6'**	GVTSAPDTRPAPG TAPPAHGVT
7	RPAPGS APPAHG	**7'**	GVTSAPDTRPAPGS APPAHGVT

The white letters highlighted in black are the amino acids bearing Tn. [a]For STD-NMR, each compound has an acetyl group at *N*-t and amide group at *C*-t. [b]For MA assay, each (glyco)peptide has a 5-oxo-hexanoyl group and a PEG linker at *N*-t and amide group at *C*-t.

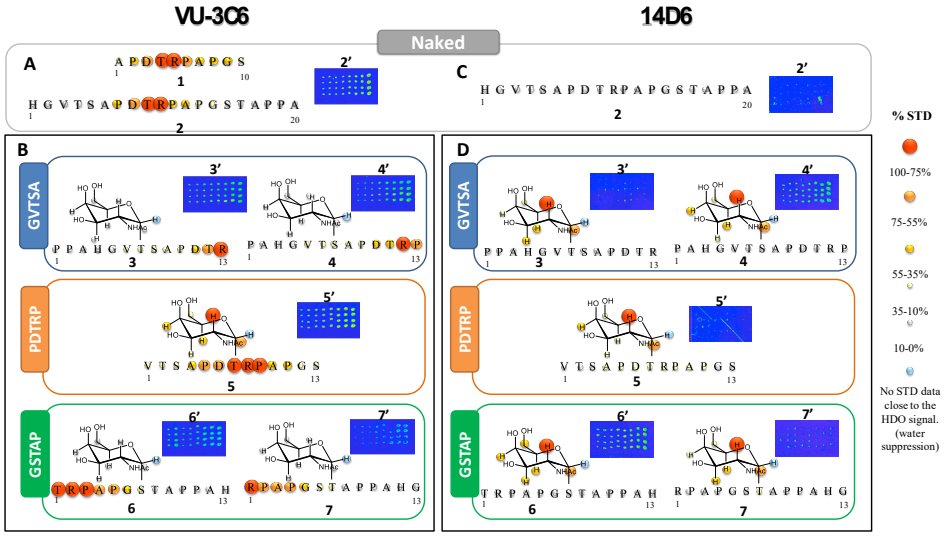

Figure 2. The STD-derived epitope and MA fluorescent scan for MUC1-derived (glyco)peptides with VU-3C6 and 14D6 mAbs (see Methods in SI). **A.** naked peptides (**1**, **2** and **2'**) with VU-3C6; **B.** glycopeptides (**3** – **7** and **3'** – **7'**) with VU-3C6; **C.** naked peptides (**1**, **2** and **2'**) with 14D6 and **D.** glycopeptides (**3** – **7** and **3'** – **7'**) with 14D6. Figure SI9-SI23 show the ^{1}H-STD spectra and additional STD-epitope representations. For MA each peptide was printed in slide at eight different concentrations incubated with the mAbs, VU-3C6 (10μg/mL) and 14D6 (200μg/mL). Figure SI3-4 and SI6-7 show MA fluorescent response graphs.

ligand-antibody complexes.[15] STD-NMR is very sensitive for weak-medium binders, as most glycan-receptor systems, and highly accurate to identify the atoms of the ligand in closer contact with the receptor.[16] In fact, STD-NMR has been employed to determine the epitope of short MUC1 glycopeptides for the breast cancer-selective SM3 mAb and for endogenous macrophage galactose-type lectin (MGL).[17] Altogether in this work, we have combined MA and STD-NMR to unveil, for the first time, the structural elements required for recognition of MUC1 tumor-associated peptides by two groups of cancer-related mAbs (Methods in SI). The first group comprise a peptide-specific mAb family, the anti-MUC1 VU-3C6 and VU-11E2 mAbs, that recognize the 12-mer GVTSAPDTRPAP of the MUC1 tandem repeat.[6] In contrast, the second family consist in anti-Tn specific mAbs, 14D6 and 8D4, generated using a synthetic Tn-based vaccine (MAG-Tn3) with a demonstrated affinity towards non-related MUC1 multi-Tn peptide structures and a positive reaction towards human cancer cell lines.[9] Therefore, a rather distinct recognition profile should be expected for each group of mAbs allowing to explore our integrated strategy as a new method to unveil the minimal key interactions, with atomic resolution, of antigen-antibody complexes. A proper MUC1 chemical library for STD and MA assays (table 1) either containing naked peptides (**1** and **2/2'**) and Tn-glycosylated in all Ser/Thr positions at the three MUC1 regions (GVTSA, PDTRP, GSTAP) has been designed (**3/3'** – **7/7'**). To expedite the synthesis of the (glyco)peptides, we employed microwave-assisted solid-phase synthesis and "double-activation" approach (Methods in SI).[18] For MA, the glycan array slide was selected due to the non-fouling surface and covalent immobilization through an oxime-bond (Methods in SI).[11,19] The mAb concentration was adjusted to facilitate optimal detection and to get comparable "relative fluorescence unit" (RFU) values among the mAbs. STD-NMR experiments were performed on 1:40 molar ratio mixtures of the mAbs in the presence of the individual compounds **1** – **8** (Methods in SI). The combined MA and STD-NMR data point out that VU-3C6 (Figure 2A and Figures SI3-4, SI9–16) and VU-11E2 (Figures SI2A and SI5) mAbs specifically bind to the non-glycosylated MUC1-derived peptides. Accordingly, the STD analysis identified the TR peptide moiety of the PDTRP region as the main structural motif for the recognition of VU-3C6 mAb (Figure

2A and Figure SI9-10), whereas VU-11E2 needs a more extended epitope involving all the PDTRP sequence (Figure SI2A).The STD-derived epitope (**1** *vs* **2**) seems to be independent of the peptide length. For glycopeptide **5** (PDTRP), the STD-NMR results disclosed that GalNAc recognition is established through the H2 proton and the *N*-acetyl moieties (Figure 2B and SI2-B). The detailed analysis of STD-NMR results for the MUC1 glycopeptides (**3** – **7**) in presence of VU-3C6 (Figure 2B) or VU-11E2 (Figure SI2-B) mAbs show up the remarkable binding selectivity for the PDTRP peptide region. For the Tn-bearing structures at the GVTSA (**3** and **4**) and GSTAP (**6** and **7**) regions of MUC1, only those amino acids present in the PDTRP fragment received saturation from the VU-3C6 mAb and GalNAc residue does not show any contact (Figure 2B). Therefore, STD-NMR seems to show a direct correlation with MA data, in which all glycopeptides manifest binding to VU-3C6 mAb, as they include the Arg residue of the region PDTR. In the case of VU-11E2 the extended PDTRP epitope region is required for a stable binding event (Figure SI2-B). Fittingly, no STD response is observed for Tn-glycopeptides at GSTAP (**6** and **7**). MA results show that the introduction of GalNAc at PDTRP region (compound **5'** *vs* **2'**) improved binding affinity, highlighting the influence of glycosylation on the tumor-specific epitope for anti-MUC1 antibodies in accordance with the significant STD signals observed in the sugar residue (Figure 2A-B) and the higher relative fluorescence response observed by MA (SI4-5).[20] Therefore, both anti-MUC1 mAbs bind GalNAc in a strict peptide-sequence-dependent manner, with a fairly specific binding profile with respect to the glycosylation position. The GalNAc-residue at PDTRP does not impedes the binding and the peptide contact epitope, deduced by STD, is the same as the observed for the naked peptides indicating the glycosylation at that position must not modified significantly the peptide conformations bound by these antibodies. (Figure 2A-B and Figure SI2A-B). Binding epitopes of the non-glycosylated PDTRP pentapeptide and the Tn-glycopentapeptide in presence of SM3 breast-cancer-related mAb was previously determined highlighting a peptide epitope concentrated at the PDT segment in the naked peptide and a more extended epitope map, whereas all PDTRP sequence is interacting, in the case of the Tn-glycopentapeptide.[17a] The 14D6 and 8D4 mAbs recognize multiple Tn-based of not correlated MUC1 peptide fragments.[9] Accordingly, MA and STD-NMR approach unequivocally demonstrates that the Tn-motif in the MUC1 sequence is required for binding (Figure 2 C and SI2 C). In addition, MA data clearly indicate that 14D6 and 8D4 display higher affinity to glycopeptides containing the Tn-Ser antigen (**4'** and **6'**) rather than the Tn-Thr (**3'**, **5'** and **7'**) alternative (Figure 2D, Figure SI2D, SI6 and SI8). By MA, these mAbs did not recognize at detectable levels the Tn-Thr glycopeptide **3'** and high concentrations of mAbs are required to detect binding of **5'** and **7'** (Figure SI7). In contrast, specific STD signals were observed for all Tn-bearing glycopeptides **3** – **7**, highlighting that both mAbs mostly recognize the GalNAc residue with a clear participation of the acetamide moiety in the binding. The peptide backbone is marginally involved in intermolecular contacts (Figure 2D and SI2D). In addition, STD-NMR unequivocally demonstrated that the monosaccharide **8** (CH_3-α-D-GalNAc) specifically binds to 8D4 and 14D6 mAbs with a similar epitope as glycopeptides **3** – **7** (Figure S23-24). A similar behavior was found for the glycopeptide-specific 237 mAb.[21] From STD competition experiments (see SI for further details) allowed to deduce that glycopeptides **3** – **7** displace GalNAc **8** from the binding site (Figure 3A and Figure SI25) as well as, that none of the glycopeptides bind with higher affinity than **8** (glycopeptide/GalNAc ratios greater than 1 do not produce reduction in GalNAc STD intensity of more than 50%) in very good agreement with STD-derived epitope. Fittingly, the analysis of the STD competition data indicated that Tn-Thr glycopeptide **3** was the weaker binder towards 14D6, probably in the low mM range. The data indicate that the 14D6 present a typical lower affinity of anti-carbohydrate antibodies that could be improved in principle by multivalence effects of a densely MUC1 Tn-antigens presentation in tumor cells.[13] The preference of this mAbs family towards Tn-Ser glycopeptides was also corroborated by STD-NMR. The STD data for **9**, displaying two simultaneous glycosylations within the MUC1 sequence (Ser at GVTSA, as in **4** and Thr at GSTAP, as in **7**), also reflects the 14D6 mAb selectivity towards glycopeptides carrying Tn-Ser in agreement with MA data. The H2 and NHAc resonance signals of GalNAc at the Ser glycosylation site received much more saturation from 14D6 than the corresponding signals at the GalNAc-Thr fragment (Figure 3B). Mazal et al. demonstrated that Ser/Thr selectivity's plays a key role for anti-Tn antibodies expression and specificity for breast and colon cancer detection.[9] Accordingly the data presented herein strongly suggests that the chemical nature of the amino acid carrier (Ser *vs* Thr) plays a key role for anti-Tn antibodies recognition. Differences in the molecular recognition features between Ser- and Thr-containing Tn antigens have been previously reported for lectins and SM3 antibody.[22]

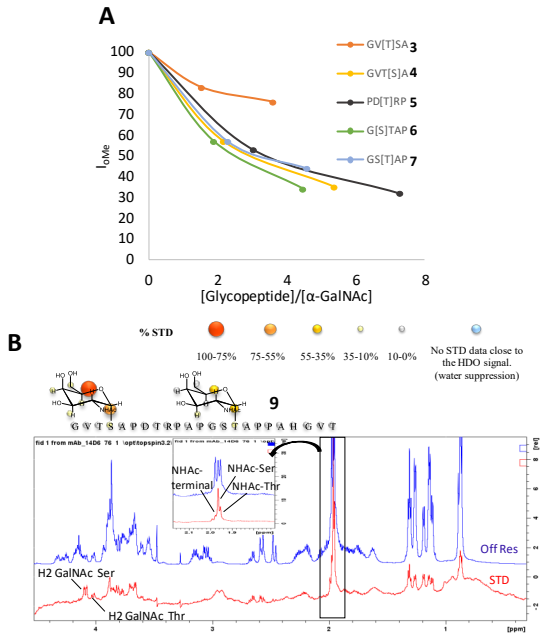

Figure 3. **A**. STD intensity of OCH3 group of **8** as function of [**3** – **7**]/[GalNAc] concentration. **B.** STD-epitope mapping and ^{1}H-STD NMR spectrum of **9** in presence of 14D6.

The conformational behavior of ligands **3** - **7** were deduced by using NOE studies (Figure S26-S30) assisted with molecular dynamics simulations with time-averaged restraints (MD-tar) highlighting that the side chain of GalNAc-Ser peptides are significantly more flexible than those containing GalNAc-Thr fragments (Figures SI32-36). The additional flexibility of Tn-Ser glycopeptides may allow to these glycopeptides to adopt the proper complementary conformation in the bound state, without a major entropy penalty. The relevance of the flanking amino acids around Ser/Thr glyco-

sylation cannot be ignored and the presentation mode of the sugar epitope is rather distinct in the Ser and Thr glycosylated peptides.

There is an interest on understanding how molecules are displayed on microarray and the contribution of the solid-support.[25] By comparison of results with the STD-NMR data, we can hypothesize that the glycopeptides presentation on glycan array slides through oxime linker was successful and can contribute to identify the specific epitopes.

A combined multidisciplinary approach, integrating synthetic chemistry methods, mAb generation, microarray, NMR and computational methods has been applied to identify the molecular elements of the recognition region of the antigens for two different families of cancer-related mAbs. The combination of MA and STD-NMR has provided a unique opportunity to investigate the functional significance of glycosylated peptides as antigens, getting detailed information for the design of tailored Tn-based vaccines like the MAG-Tn3. Our results highlight that for anti-MUC1 mAbs, the amino acids sequence region modulates the affinity of the mAb, while for anti-Tn mAbs, it is the type of residue that modulates the binding. The integrated methodology reported herein, can be employed, in general, to study antigen-antibody interactions, and will be of paramount importance to designing a potent multivalent Tn-MUC1 synthetic anticancer vaccine that raises functional antibodies against tumor-associated antigens.

ASSOCIATED CONTENT

Supporting Information

Methods and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

filipa.marcelo@fct.unl.pt.
faynagarciam@glyco.sci.hokudai.ac.jp

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

The authors thank FCT-Portugal for the financial support of EXPL/QEQ/MED/0799/2012, RECI/BBB-BQB/0230/2012 and UID/Multi/04378/2013 and the Ministry of Education, Culture, Science, and Technology, Japan for Grant Nº 25220206. F.M. thanks FCT for the grant (SFRH/BPD/65462/2009) F.G.M. acknowledges JSPS Wakate B KAKENHI Grant Nº 24710242. G.A. thanks Canon Foundation. We thank Ms. S. Oka, and T. Hirose at the Center for Instrumental Analysis, Hokkaido University. C.L. thanks the Ligue Nationale Contre le Cancer (Equipe Labellisée 2014) and Banque Privée Européenne for the financial support.

REFERENCES

(1) Hattrup, C. L.; Gendler, S. J. *Annu Rev Physiol* **2008**, *70*, 431.
(2) (a) Hollingsworth, M. A.; Swanson, B. J. *Nat Rev Cancer* **2004**, *4*, 45. (b) Dube, D. H.; Bertozzi, C. R. *Nat Rev Drug Discov* **2005**, *4*, 477.
(3) Gaidzik, N.; Westerlind, U.; Kunz, H. *Chem Soc Rev* **2013**, *42*, 4421.
(4) Richichi, B.; Thomas, B.; Fiore, M.; Bosco, R.; Qureshi, H.; Nativi, C.; Renaudet, O.; BenMohamed, L. *Angew Chem Int Ed Engl* **2014**, *53*, 11917.
(5) Lo-Man, R.; Vichier-Guerre, S.; Perraut, R.; Deriaud, E.; Huteau, V.; BenMohamed, L.; Diop, O. M.; Livingston, P. O.; Bay, S.; Leclerc, C. *Cancer Res* **2004**, *64*, 4987.
(6) (a) Schol, D. J.; Meulenbroek, M. F.; Snijdewint, F. G.; Mensdorff-Pouilly, von, S.; Verstraeten, R. A.; Murakami, F.; Kenemans, P.; Hilgers, J. *Tumor Biol* **1998**, *19 Suppl 1*, 35. (b) Blixt, O.; Lavrova, O. I.; Mazurov, D. V.; Clo, E.; Kracun, S. K.; Bovin, N. V.; Filatov, A. V. *Glycobiology* **2012**, *22*, 529.
(7) (a) Karsten, U.; Diotel, C.; Klich, G.; Paulsen, H.; Goletz, S.; Müller, S.; Hanisch, F. G. *Cancer Res* **1998**, *58*, 2541. (b) Mensdorff-Pouilly, von, S.; Petrakou, E.; Kenemans, P.; van Uffelen, K.; Verstraeten, A. A.; Snijdewint, F. G.; Van Kamp, G. J.; Schol, D. J.; Reis, C. A.; Price, M. R.; Livingston, P. O.; Hilgers, J. *Int J Cancer* **2000**, *86*, 702.
(8) Grinstead, J. S.; Koganty, R. R.; Krantz, M. J.; Longenecker, B. M.; Campbell, A. P. *Biochemistry* **2002**, *41*, 9946.
(9) Mazal, D.; Lo-Man, R.; Bay, S.; Pritsch, O.; Deriaud, E.; Ganneau, C.; Medeiros, A.; Ubillos, L.; Obal, G.; Berois, N.; Bollati-Fogolin, M.; Leclerc, C.; Osinaga, E. *Cancer Immunol* **2013**, *62*, 1107.
(10) (a) Fukui, S.; Feizi, T.; Galustian, C.; Lawson, A. M.; Chai, W. *Nat Biotechnol* **2002**, *20*, 1011. (b) Smith, D. F.; Song, X.; Cummings, R. D. In *Methods in Enzymology*; Use of Glycan Microarrays to Explore Specificity of Glycan-Binding Proteins. (Ed.: M. Fukuda) Elsevier, **2010**, *480*, pp. 417–444. (c) Foong, Y. M.; Fu, J.; Yao, S. Q.; Uttamchandani, M. *Curr Opin Chem Biol* **2012**, *16*, 234.
(11) Matsushita, T.; Takada, W.; Igarashi, K.; Naruchi, K.; Miyoshi, R.; García-Martín, F.; Amano, M.; Hinou, H.; Nishimura, S.-I. *Biochim Biophys Acta* **2014**, *1840*, 1105.
(12) (a) Oyelaran, O.; Li, Q.; Farnsworth, D.; Gildersleeve, J. C. *J Proteome Res*, **2009**, *8*, 3529. (b) Borgert, A.; Heimburg-Molinaro, J.; Song, X.; Lasanajak, Y.; Ju, T.; Liu, M.; Thompson, P.; Ragupathi, G.; Barany, G.; Smith, D.F.; Cummings, R.D.; Live, D. *Chem Biol* **2012**, *7*, 1031. (c) Cai, H.; Palitzsch, B.; Hartmann, S.; Stergiou, N.; Kunz, H.; Schmitt, E.; Westerlind, U. *Chembiochem* **2015**, *16*, 959.
(13) Haji-Ghassemi, O.; Blackler, R. J.; Young, N. M.; Evans, S. V. *Glycobiology* **2015**
(14) Agirre, J.; Davies, G.; Wilson, K.; Cowtan, K. *Nat Chem Biol* **2015**, *11*, 303.
(15) Marcelo, F.; Cañada, F. J.; Jimenez-Barbero, J. In *Anticarbohydrate Antibodies*; In Anticarbohydrate antibodies: from molecular basis to clinical application. (Ed.: P. Kosma, S. Müller-Leonnies), Springer-Verlag, Wien.: Vienna, **2012**; pp. 385–402 and references cited herein.
(16) Meyer, B.; Peters, T. *Angew Chem* **2003**, *115*, 890; *Angew Chem Int Ed Engl* **2003**, *42*, 864
(17) (a) Möller, H.; Serttas, N.; Paulsen, H.; Burchell, J. M.; Taylor-Papadimitriou, J.; Meyer, B. *Eur J Biochem* **2002**, *269*, 1444 -(b) Marcelo, F.; García-Martín, F.; Matsushita, T.; Sardinha, J.; Coelho, H.; Oude-Vrielink, A.; Koller, C.; André, S.; Cabrita, E. J.; Gabius, H.-J.; Nishimura, S.-I.; Jimenez-Barbero, J.; Cañada, F. J *Chem Eur J* **2014**, *20*, 16147.
(18) (a) Matsushita, T.; Hinou, H.; Kurogochi, M.; Shimizu, H.; Nishimura, S.-I. *Org Lett* **2005**, *7*, 877. (b) García-Martín, F.; Hinou, H.; Matsushita, T.; Hayakawa, S.; Nishimura, S.-I. *Org Biomol Chem* **2012**, *10*, 1612.
(19) García-Martín, F.; Matsushita, T.; Hinou, H.; Nishimura, S.-I. *Chem. Eur. J.* **2014**, *20*, 15891.
(20) Karsten, U.; Serttas, N.; Paulsen, H.; Danielczyk, A.; Goletz, S. *Glycobiology* **2004**, *14*, 681.
(21) Brooks, C. L.; Schietinger, A.; Borisova, S. N.; Kufer, P.; Okon, M.; Hirama, T.; Mackenzie, C. R.; Wang, L.-X.; Schreiber, H.; Evans, S. V. *Proc Natl Acad Sci USA* **2010**, *107*, 10056.
(22) (a) Madariaga, D.; Martínez-Sáez, N.; Somovilla, V. J.; García-García, L.; Berbis, M. Á.; Valero-Gonzalez, J.; Martín-Santamaría, S.; Hurtado-Guerrero, R.; Asensio, J. L.; Jimenez-Barbero, J.; Avenoza, A.; Busto, J. H.; Corzana, F.; Peregrina, J. M. *Chem. Eur. J.* **2014**, *20*, 12616. (b) Martínez-Sáez, N.; Castro-López, J. Valero-González, J.; *et al. Angew Chem Int Ed* **2015**, *54*, 9830.
(25) Wang, L.; Cummings, R. D.; Smith, D. F.; Huflejt, M.; Campbell, C. T.; Gildersleeve, J. C.; Gerlach, J. Q.; Kilcoyne, M.; Joshi, L.; Serna, S.; Reichardt, N.-C.; Parera Pera, N.; Pieters, R. J.; Eng, W.; Mahal, L. K. *Glycobiology* **2014**, *24*, 507.

Insert Table of Contents artwork here